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KEEPING QUALITIES OF MARKET SAMPLES OF NEOARSPHENAMINE WHILE IN AMPULE.

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Comparatively little is known concerning the keeping qualities of market samples of the newer organic arsenicals, arsphenamine and neoarsphenamine.[1]

Both compounds are regarded as very unstable substances when exposed to the air, and for this reason they are prepared for the market in sealed glass containers from which the air has been excluded either by production of a vacuum or by displacement of the air with a nonoxidizing gas such as carbon dioxide, nitrogen, or hydrogen.[2]

During the last three years many lots of commercial arsphenamine and neoarsphenamine, comprising samples from practically every domestic manufacturer, were submitted to the Hygienic Laboratory for reexamination, and in no instance was a lot of arsphenamine encountered which could be definitely said to have deteriorated. However, a number of lots of neoarsphenamine were found which showed deterioration since their first examination.[3]

Many preparations of neoarsphenamine were enountered in which striking changes were noticed in many of their physicochemical properties, such as changes in color, solubility, mobility in ampule, and odor. No instances of such changes were found in any of the arsphenamines examined.

[1] Throughout this paper the official names arsphenamine and neoarsphenamine are used to designate the compounds formerly known exclusively as salvarsan and neosalvarsan, respectively, but now being manufactured under various trade names. Arsphenamine is the dihydrochloride of 3, 3'-diamino-4, 4'-dihydroxyarsenobenzene, while neoarsphenamine is usually considered to be sodium 3, 3'-diamino-4, 4' dihydroxyarsenobenzene-n-methylene sulphinate.

[2] See U. S. Patent No. 1,081,592, P. Ehrlich and A. Bertheim, Dec. 16, 1913.

[3] According to an act of Congress (32 Stat., 728) and regulations issued by the Secretary of the Treasury, all arsphenamine and allied products must be examined by the Federal Government before being released for interstate or foreign trade. Such examination is made in the Hygienic Laboratory of the United States Public Health Service and consists of both chemical and toxicological tests. Briefly, the regulations require that in the toxicological tests, white rats weighing between 100 and 150 grams shall live 48 hours when given arsphenamine intravenously as a 2 per cent alkaline aqueous solution, in dosage of 100 mgm. per kilo, 0.9 c. c. of normal sodium hydroxide being used to alkalinize each 0.1 gm. of arsphenamine. White rats of similar weights are required to live 7 days when given neoarsphenamine as a 4 per cent aqueous solution in dosage of 200 mgm. per kilo. The rate of injection for both compounds shall be from 12 to 15 seconds for each 0.1 c. c. of fluid. The details of the tests are to be found in Miscellaneous Publication No. 22 of the United States Public Health Service, 1920.

It thus appeared that arsphenamine was the more stable of the two compounds and that further investigation of the keeping qualities of neoarsphenamine was especially imperative. A more extensive investigation was therefore made, and the results are reported herein.

The present report deals almost wholly with products from four of the largest domestic manufacturers, and the age of the preparations in no case was over three years. The examination included tests for toxicity, made according to the above-described official method, and observations on changes in solubility, mobility in ampule, color, and odor.

The changes in solubility were determined by making up a 4 per cent solution of the drug in distilled water. The drug was considered incompletely soluble if, when made up in the above manner, a clear solution was not effected within 10 minutes. It was considered that no increase in toxicity had occurred unless more than 40 per cent of the animals reacted differently from the manner in which they reacted at the first examination. For example, if a sample killed 1 out of 5 rats at a certain dosage at first examination, and on second examination killed 2 out of 5 at the same dosage, no difference in toxicity was recorded. If, however, 3 or more died out of 5 on second examination, the preparation was considered to be more toxic than at first examination.

Throughout this paper room temperature refers to temperatures ranging from 20° to 29° centigrade (inclusive), and storeroom temperature to variations of from 18° to 20° centigrade (inclusive). All storeroom samples were kept in the dark in closed packages.

The major portion of the experiments are tabulated in Tables I, II, III, and IV, and respectively represent products from four different domestic manufacturers.

The official observation period of seven days was used in obtaining the results of the toxicity tests referred to above.

TABLE I.—*Keeping qualities of neoarsphenamine (manufacturer A) while in ampule and when subjected to various conditions, toxicity being indicated by the tolerance shown by white rats, when administered intravenously as a 4 per cent aqueous solution.*

Sample.	Date of manufacture.	How kept.	Appearance and physical properties at examination.	Date of examination.	Details and results of examination.	Remarks.
1	March, 1919.....	Room temp. (20°-29° C.).	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	March, 1919.....	1 died on fifth day, out of 5 injected at 200 mgm. per kilo.	
1	.....do.....	Storeroom temp. (18°-20° C.).	Deep golden yellow powder; freely mobile in ampule; turbid solution in water at room temp.; alkaline to litmus.	March, 1920.....	No deaths out of 5 injected at 200 mgm. per kilo.	Solution filtered.
2	.....do.....	Room temp.....	Light golden yellow powder; readily soluble in water at room temp.; freely mobile in ampule.	July, 1919.....	1 died on fifth day, out of 5 injected at 240 mgm. per kilo; 3 died within 4 days, out of 10 injected at 280 mgm. per kilo.	Test in March: 1 died on first day, out of 5 injected at 200 mgm. per kilo.
2	.....do.....	Storeroom temp.....	Light golden yellow powder; freely mobile in ampule; sl. turbid solution in water at room temp.	March, 1921.....	1 death on first day, out of 5 injected at 240 mgm. per kilo (4 per cent solution).	Garlicky odor to solution; turbid after 20 minutes.
3	.....do.....	Room temp.....	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	.....do.....	No deaths out of 5 injected at 200 mgm. per kilo.	
3	.....do.....	Submitted for reexamination; probably kept at room temp.	Light golden yellow powder; freely mobile in ampule; turbid solution after ½ hour in water at room temp.	July, 1920.....	.....do.....	Solution still turbid after filtration; 2 animals received filtered, 3 unfiltered solution.
4	November, 1919..	Room temp.....	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	November, 1919..	.....do.....	Solution alkaline to litmus.
4	.....do.....	Storeroom temp.....	Light golden yellow powder; freely mobile in ampule; incompletely soluble; fine flocculent suspension; alkaline to litmus.	March, 1920.....	1 died on fourth day, out of 5 injected at 200 mgm. per kilo.	Solution filtered; alkaline to litmus.
5	December, 1919..	Room temp.....	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	December, 1919..	No deaths out of 5 injected at 200 mgm. per kilo.	Injection made in 5 to 6 seconds.
5	.....do.....	Submitted for reexamination; probably kept at room temp.	Light golden yellow powder; freely mobile in ampule; difficultly soluble in water at room temp.; complete solution after 20 minutes.	July, 1920.....	1 death out of 5 injected at 200 mgm. per kilo.	Storeroom sample also difficultly soluble; powder trifle darker than submitted.
6	January, 1920....	Room temp.....	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	January, 1920....	2 deaths—1 on fourth, 1 on fifth day—out of 10 injected at 200 mgm. per kilo.	
6	.....do.....	Storeroom temp.....	.....do.....	June, 1921.....	No deaths out of 10 injected at 200 mgm. per kilo.	
7	.....do.....	Room temp.....	.....do.....	January 12, 1920.	3 died out of 5 injected at 200 mgm. per kilo.	

TABLE I.—*Keeping qualities of neoarsphenamine (manufacturer A) while in ampule and when subjected to various conditions, toxicity being indicated by the tolerance shown by white rats, when administered intravenously as a 4 per cent aqueous solution*—Continued.

Sample.	Date of manufacture.	How kept.	Appearance and physical properties at examination.	Date of examination.	Details and results of examination.	Remarks.
7	January, 1920	Room temp	Light golden yellow powder; freely mobile in ampule; slightly turbid solution after ½ hour.	January, 23, 1920.	4 died out of 5 injected at 200 mgm. per kilo.	Withdrawn by manufacturer.
7	do	Storeroom temp	do	July, 1921	1 died within 7 days out of 5 injected at 200 mgm. per kilo.	Neutral to litmus; solution not filtered.
8	February, 1920	Room temp	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	April, 1920	1 died out of 5 injected at 420; 5 died out of 5 injected at 500 mgm. per kilo.	500 mgm. dose given as 10 per cent solution; 420 mgm. dose given as 4 per cent solution.
8	do	Storeroom temp	do	March, 1921	4 died within 4 days, out of 5 injected at 400 mgm. per kilo.	Given as 10 per cent solution.
8	do	do	do	do	3 died within 4 days, out of 5 injected at 300 mgm. per kilo; none died out of 5 injected at 200 mgm.	4 per cent solution.
8	do	do	do	June, 1921	7 died within 1 day, out of 10 injected at 400 mgm. per kilo.	10 per cent solution.
9	April, 1920	Room temp	do	April, 1920	No deaths out of 5 injected at 200 mgm. per kilo.	
9	do	Storeroom temp	do	July, 1921	do	
10	July, 1920	Room temp	do	August, 1920	None died out of 5 injected at 200; 2 died within 5 days, out of 5 at 300; and 5 within 3 days, out of 5 injected at 400 mgm. per kilo.	Rats on 200 mgm. dose received 4 per cent solution; all others 10 per cent solution.
10	do	Storeroom temp	do	March, 1921	1 died on fifth day, out of 5 injected at 200 mgm. per kilo.	4 per cent solution.
11	do	Room temp	do	July, 1920	No deaths out of 5 injected at 200 mgm. per kilo.	
11	do	Submitted for reexamination; probably kept at room temp.	do	May, 1921	do	
12	August, 1920	Room temp	do	August, 1920	1 death on third day, out of 5 injected at 400; 5 deaths within 24 hours, out of 5 injected at 500 mgm. per kilo.	Given as 10 per cent solution.
12	do	Storeroom temp	do	March, 1921	5 died within 2 days, out of 5 injected at 400 mgm. per kilo; no deaths at 200 mgm., 5 injected.	200 mgm. dose given as 4 per cent solution; 400 mgm. as 10 per cent solution.
12	do	do	do	June, 1921	4 died within 5 days, out of 5 injected at 400 mgm. per kilo.	10 per cent solution.
13	do	Room temp	do	August, 1920	2 died within 2 days, out of 10 injected at 200 mgm. per kilo.	
13	do	Storeroom temp	do	June, 1921	None died out of 10 injected at 200 mgm. per kilo.	

TABLE II.—*Keeping qualities of neoarsphenamine (manufacturer B) while in ampule and when subjected to various conditions, toxicity being indicated by the tolerance shown by white rats, when administered intravenously as a 4 per cent solution.*

Sample.	Date of manufacture.	How kept.	Appearance and physical properties at examination.	Date of examination.	Details and results of examination.	Remarks.
1	December, 1918...	Room temp. (20°–29° C.).	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	December, 1918...	3 died within 6 days, out of 10 injected at 180 mgm. per kilo.	
1	.....do.....	Submitted for reexamination; probably kept at room temp.	Deep golden yellow powder; adheres slightly to sides of ampule; incompletely soluble in water at room temp.	May, 1921.....	No deaths out of 5 injected at 200 mgm. per kilo.	
2	February, 1919...	Room temp.....	Deep golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	March, 1919.....	No deaths out of 5 injected at 200; 8 deaths within 4 days, out of 10 injected at 240 mgm. per kilo.	
2	.....do.....	.....do.....	Deep golden yellow powder; freely mobile in ampule; forms turbid solution.	May, 1921.....	2 deaths within 6 days, out of 5 injected at 200 mgm. per kilo.	
3	May, 1919.....	.....do.....	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	May, 1919.....	2 deaths within 7 days, out of 10 injected at 200 mgm. per kilo.	
3	.....do.....	Storeroom temp. (18°–20° C.).	.....do.....	June, 1921.....	No deaths out of 10 injected at 200 mgm. per kilo.	
4	June, 1919.....	Room temp.....	.....do.....	June, 1919.....	No deaths out of 5 injected at 200 mgm. per kilo.	
4	.....do.....	Submitted for reexamination; probably kept at room temp.	Light golden yellow; freely mobile in ampule; incompletely soluble in water at room temp.; sharp garlicky odor to solution.	December, 1919...	.....do.....	Solution unfiltered.
4	.....do.....	.....do.....	.....do.....	June, 1920.....	1 death on first day, out of 5 injected at 200 mgm. per kilo.	Death occurred from unfiltered solution.
5	September, 1919..	Room temp.....	Deep golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	September, 1919..	1 death on third day, out of 5 injected at 200 mgm. per kilo.	
5	.....do.....	Submitted for reexamination; probably kept at room temp.	.....do.....	May, 1920.....	No deaths out of 5 injected at 200 mgm. per kilo.	
5	.....do.....	Storeroom temp.....	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	July, 1921.....	.....do.....	
6	December, 1919 ..	Room temp.....	.....do.....	December, 1919 ..	4 deaths within 5 days, out of 10 injected at 200 mgm. per kilo.	
6	.....do.....	Submitted for reexamination; probably kept at room temp.	Deep golden yellow powder; freely mobile in ampule; incompletely soluble in water at room temp.	April, 1920.....	No deaths out of 5 injected at 200 mgm. per kilo.	Solution filtered.
7	January, 1920....	Room temp.....	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	January, 1920....	1 death on fourth day, out of 5 injected at 200 mgm. per kilo.	

TABLE II.—*Keeping qualities of neoarsphenamine (manufacturer B) while in ampule and when subjected to various conditions, toxicity being indicated by the tolerance shown by white rats, when administered intravenously as a 4 per cent solution*—Continued.

Sample.	Date of manufacture.	How kept.	Appearance and physical properties at examination.	Date of examination.	Details and results of examination.	Remarks.
7	January, 1920	Submitted for reexamination; probably kept at room temp.	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	July, 1920	No deaths out of 5 injected at 200 mgm. per kilo.	
7	do	do	do	October, 1920	1 death on fourth day, out of 5 injected at 200 mgm. per kilo.	
7	do	Storeroom temp.	do	July, 1921	No deaths out of 5 injected at 200 mgm. per kilo.	Solution alkaline to litmus.
8	April, 1920	Room temp.	do	April, 1920	3 died within 4 days, out of 10 injected at 200 mgm. per kilo.	
8	do	Storeroom temp.	do	June, 1921	1 died on first day, out of 10 injected at 200 mgm. per kilo.	
9[1]	May, 1920	Room temp.	do	May, 1920	No deaths out of 5 injected at 200 mgm. per kilo.	
9[1]	do	Storeroom temp.	Orange-red powder; freely mobile in ampule; incompletely soluble in water at room temp.	July, 1921	do	Odor of ether on opening ampule.
9[2]	do	do	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	do	2 deaths within 4 days, out of 5 injected at 200 mgm. per kilo.	Suggestion of turbidity after standing.
10	August, 1920	Room temp.	do	August, 1920	No deaths out of 5 injected at 200; 1 death out of 5 injected at 200 mgm. per kilo.	Animal that died was pregnant.
10	do	Storeroom temp.	do	June, 1921	4 deaths within 6 days out of 5 injected at 200; 5 deaths within 3 days out of 5 injected at 300 mgm. per kilo.	
11	September, 1920	Room temp.	do	September, 1920	No deaths out of 5 injected at 200 mgm. per kilo.	
11	do	Submitted for reexamination; probably kept at room temp.	do	May, 1921	do	
12	do	Room temp.	do	September, 1920	do	
12	do	Submitted for reexamination; probably kept at room temp.	do	May, 1921	2 deaths within 4 days, out of 5 injected at 200 mgm. per kilo.	
13	October, 1920	Room temp.	do	October, 1920	4 deaths within 7 days, out of 10 injected at 200 mgm. per kilo.	
13	do	Storeroom temp.	do	June, 1920	do	
14	do	Room temp.	do	November, 1920	3 deaths within 6 days, out of 10 injected at 200 mgm. per kilo.	Odor of ether on opening ampule.
14	do	Storeroom temp.	do	June, 1921	None died out of 10 injected at 200 mgm. per kilo.	

[1] Shipment 1.

[2] Shipment 2.

TABLE III.—*Keeping qualities of neoarsphenamine (manufacturer C) while in ampule and when subjected to various conditions, toxicity being indicated by the tolerance shown by white rats, when administered intravenously as a 4 per cent aqueous solution.*

Sample.	Date of manufacture.	How kept.	Appearance and physical properties at examination.	Date of examination.	Details and results of examination.	Remarks.
1	February, 1919...	Room temp. (20°–29° C.)..	Golden yellow powder: freely mobile in ampule; readily soluble in water at room temp.	February, 1919...	1 death on first day, out of 5 injected at 200 mgm. per kilo.	Odor of ether on opening ampule.
1	.....do.....	Storeroom temp. (18°–20° C.).	Brick red cake; can not be moved on shaking ampule; practically insoluble in water at room temp.	March, 1921.....	No deaths out of 5 injected at 200 mgm. per kilo (filtrate contained 0.75 per cent As).	Odor of ether on opening ampule; material settles.
2	.....do.....	Room temp.....	Golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	February, 1919...	1 death out of 5 injected at 200 mgm. per kilo.	Odor of ether on opening ampule.
2	.....do.....	Storeroom temp.....	Orange red powder; freely mobile in ampule; readily soluble in water at room temp.; garlicky odor to solution.	March, 1921.....	No deaths out of 5 injected at 200 mgm. per kilo.	
3	May, 1919.....	Room temp.....	Golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	May, 1919.....	.....do.....	Marked odor of ether on opening ampule.
3	.....do.....	Storeroom temp.....	Brick-red powder; freely mobile in ampule; incompletely soluble in water at room temp.; coarse suspension.	March, 1921.....	1 death on second day, out of 5 injected at 200 mgm. per kilo.	Do.
4	.....do.....	Room temp.....	Golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	May, 1919.....	No deaths out of 5 injected at 200 mgm. per kilo.	Odor of ether on opening ampule.
4	.....do.....	Storeroom temp.....	Brick-red powder; freely mobile in ampule; incompletely soluble in water at room temp.	March, 1921.....	.....do.....	Solution alkaline to litmus.
5	June, 1919.....	.....do.....	Deep golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	July, 1919.....	2 died within 5 days, out of 10 injected at 200; 4 within 7 days out of 10 injected at 240; 9 within 4 days out of 10 injected at 320 mgm. per kilo.	
5	.....do.....	.....do.....	.....do.....	May, 1920.....	1 died on second day, out of 5 injected at 200 mgm. per kilo.	Odor of ether on opening ampule.
5	.....do.....	Room temp.; exposed to light (sunlight part of time) since Aug. 12, 1919.	.....do.....	June, 1920.....	No deaths out of 5 injected at 200 mgm. per kilo.	Slight garlicky odor to solution.
5	.....do.....	Incubated at 37° C. since Sept. 19, 1919.	.....do.....	.....do.....	.....do.....	Do.
5	.....do.....	Storeroom temp.....	.....do.....	.....do.....	.....do.....	
5	.....do.....	.....do.....	.....do.....	February, 1921...	1 died on seventh day, out of 5 injected at 200 mgm. per kilo.	Garlicky odor to solution.

TABLE III.—*Keeping qualities of neoarsphenamine (manufacturer C) while in ampule and when subjected to various conditions, toxicity being indicated by the tolerance shown by white rats, when administered intravenously as a 4 per cent aqueous solution*—Continued.

Sample.	Date of manufacture.	How kept.	Appearance and physical properties at examination.	Date of examination.	Details and results of examination.	Remarks.
5	June, 1919.....	Storeroom temp.....	Deep golden yellow powder; freely mobile in ampule; readily soluble in water ot room temp.	June, 1921.....	1 death on fifth day, out of 5 injected at 200 mgm. per kilo.	Odor of ether on opening ampule.
5	.....do.....	Incubated at 37° C. since Sept. 19, 1919.	Reddish powder; freely mobile in ampule; incompletely soluble in cold water; partial solution contained much flocculent material.	...do.....	No deaths out of 5 injected at 200 mgm. per kilo.	Do.
6	July, 1919.....	Room temp.....	Deep golden yellow; freely mobile in ampule; readily soluble in water at room temp.	July, 1919.....	.....do.....	
6	.....do.....	Storeroom temp.....	.....do.....	March, 1921.....	.....do.....	
7	September, 1919..	Room temp.....	.....do.....	September, 1919..	2 deaths within 3 days, out of 10 injected at 200 mgm. per kilo.	
7	.....do.....	Storeroom temp.....	.....do.....	March, 1921.....	4 deaths within 4 days, out of 5 injected at 200 mgm. per kilo.	
7	.....do.....	.....do.....	.....do.....	June, 1921.....	3 deaths within 5 days, out of 10 injected at 200 mgm. per kilo.	
8	April, 1920.....	Room temp.....	.....do.....	April, 1920.....	No deaths out of 5 injected at 200 mgm. per kilo.	
8	.....do.....	Storeroom temp.....	.....do.....	March, 1921.....	4 deaths within 4 days, out of 5 injected at 200 mgm. per kilo.	Odor of ether on opening ampule.
8	.....do.....	.....do.....	.....do.....	June, 1921.....	1 death on second day, out of 5 injected at 200 mgm. per kilo.	
9	September, 1920..	Room temp.....	.....do.....	September, 1920..	3 deaths within 6 days, out of 10 injected at 200 mgm. per kilo.	
9	.....do.....	.....do.....	.....do.....	June, 1921.....	1 died on third day, out of 10 injected at 200 mgm. per kilo.	
10	.....do.....	.....do.....	.....do.....	October, 1920.....	6 deaths within 7 days, out of 15 injected at 200 mgm. per kilo.	
10	.....do.....	.....do.....	.....do.....	March, 1921.....	4 deaths within 6 days, out of 5 injected at 200 mgm. per kilo.	
10	.....do.....	.....do.....	.....do.....	June, 1921.....	1 death on fourth day, out of 10 injected at 200 mgm. per kilo.	

TABLE IV.—*Keeping qualities of neoarsphenamine (manufacturer D) while in ampule and when subjected to various conditions, toxicity being indicated by the tolerance shown by white rats, when administered intravenously as a 4 per cent aqueous solution.*

Sample.	Date of manufacture.	How kept.	Appearance and physical properties at examination.	Date of examination.	Details and results of examination.	Remarks.
1	May, 1919	Storeroom temp. (18°-20° C.).	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	June, 1919	None died out of 10 injected at 200 mgm. per kilo.	
1	do	do	Light golden yellow powder; freely mobile in ampule; turbid solution in water at room temp.	March, 1921	7 died out of 10 injected at 200 mgm. per kilo.	
1	do	do	do	May, 1921	3 died within 4 days out of 5 injected at 200 mgm. per kilo.	
1	do	do	do	June, 1921	None died out of 10 injected at 200 mgm. per kilo.	
2	July, 1919	Room temp. (20°-29° C.).	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	July, 1919	1 death on sixth day, out of 5 injected at 200 mgm. per kilo.	
2	do	Submitted for reexamination; probably kept at room temp.	do	October, 1919	1 death on fourth day, out of 5 injected at 200 mgm. per kilo.	
2	do	Storeroom temp.	Light golden yellow powder; freely mobile in ampule; turbid solution in water at room temp.	July, 1921	3 died within 3 days out of 5 injected at 200 mgm. per kill.	Not filtered.
3	September, 1919	Room temp.	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	September, 1919	No deaths out of 5 injected at 200 mgm. per kilo.	
3	do	Incubated 24 hours at 37° C.	do	October, 1919	1 death on fifth day out of 5 injected at 200 mgm. per kilo.	
3	do	Storeroom temp. since September, 1919.	do	June, 1920	No deaths out of 5 injected at 200 mgm. per kilo.	
3	do	Incubated at 37° C. since Sept. 30, 1919.	Light golden yellow powder; freely mobile in ampule; incompletely soluble in water at room temp.; garlicky odor to incomplete solution.	do	do	Incomplete solution after ½ hour; filtered before injection.
3	do	Storeroom temp.	Light golden yellow powder; freely mobile; readily soluble in water at room temp.	July, 1921	2 died within 7 days out of 5 injected at 200 mgm. per kilo.	
4	do	Room temp.	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	September, 1919	No deaths out of 5 injected at 200 mgm. per kilo.	
4	do	Incubated at 37° C. for 24 hrs.	do	October, 1919	do	
4	do	Storeroom temp.	do	June, 1920	1 death on fourth day out of 5 injected at 200 mgm. per kilo.	

TABLE IV.—*Keeping qualities of neoarsphenamine (manufacturer D) while in ampule and when subjected to various conditions, toxicity being indicated by the tolerance shown by white rats, when administered intravenously as a 4 per cent aqueous solution*—Continued.

Sample.	Date of manufacture.	How kept.	Appearance and physical properties at examination.	Date of examination.	Details and results of examination.	Remarks.
4	September, 4919..	Incubated at 37° C. since Sept. 30, 1919.	Light golden yellow powder; freely mobile in ampule; incompletely soluble in water at room temp.; garlicky odor to incomplete solution; powder trifle darker than previous tube.	June, 1920.....	1 death on third day out of 5 injected at 200 mgm. per kilo.	Incomplete solution after ½ hour; filtered before injection.
4	.....do.....	Storeroom temp.....	Light golden yellow powder; freely mobile in ampule; incompletely soluble in water at room temp.; no garlicky odor to solution.	June, 1921.....	No deaths out of 5 injected at 200 mgm. per kilo.	Incomplete solution on filtered.
4	.....do.....	Incubated at 37° C. since Sept. 30, 1919.	Deep golden yellow powder; not freely mobile in ampule; odor of ether on opening ampule; incompletely soluble in cold water; much flocculent material remains undissolved; garlicky odor to solution.	.....do.....	.....do.....	Do.
5	October, 1919.....	Room temp.....	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	October, 1919.....	2 died within 4 days, out of 10 injected at 200 mgm. per kilo.	
5	.....do.....	Storeroom temp.....	.....do.....	March, 1921.....	None died out of 10 injected at 200 mgm. per kilo.	
6	.....do.....	Room temp.....	.....do.....	November, 1919..	.....do.....	
6	.....do.....	Submitted for reexamination; probably kept at room temp.	.....do.....	March, 1920.....	1 died on third day, out of 5 injected at 200 mgm. per kilo.	
6	.....do.....	Storeroom temp.....	.....do.....	July, 1921.....	No deaths out of 5 injected at 200 mgm. per kilo.	
7	March, 1920.....	Room temp.....	.....do.....	March, 1920.....	2 died on fifth day, out of 10 injected at 200 mgm. per kilo.	
7	.....do.....	Storeroom temp.....	.....do.....	March, 1921.....	2 died within 4 days, out of 5 injected at 200 mgm. per kilo.	
7	.....do.....	.....do.....	.....do.....	June, 1921.....	1 died on second day, out of 5 injected at 200 mgm. per kilo.	
8	June, 1920.....	Room temp.....	.....do.....	June, 1920.....	No deaths out of 10 injected at 200 mgm. per kilo.	
8	.....do.....	Storeroom temp.....	Light golden yellow powder; freely mobile in ampule; incompletely soluble in water at room temperature; fine flocculent ppt.	March, 1921.....	9 died within 5 days, out of 10 injected at 200 mgm. per kilo.	
8	.....do.....	.....do.....	.....do.....	June, 1921.....	All died within 6 days, out of 10 injected at 200 mgm. per kilo.	Solution not filtered; formed white scum on surface after 1 hour.

9	July, 1920	Room temp	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	July, 1920	3 died within 5 days, out of 10 injected at 200 mgm. per kilo.
9	do	Submitted for reexamination; probably kept at room temp.	Deep golden yellow powder; freely mobile in ampule; incompletely soluble in water at room temp.	June, 1921	3 died within 3 days, out of 5 injected at 200 mgm. per kilo.
9	do	Storeroom temp	Sample trifle lighter in color than previous ampule, otherwise same (insoluble).	do	4 died within 5 days, out of 5 injected at 200 mgm. per kilo.
10	November, 1920	Room temp	Light golden yellow powder; freely mobile in ampule; readily soluble in water at room temp.	December, 1920	2 deaths within 5 days, out of 10 injected at 200 mgm. per kilo.
10	do	Storeroom temp	do	March, 1921	No deaths out of 5 injected at 200 mgm. per kilo.
10	do	do	do	July, 1921	do

Summary of Results.

The foregoing tables show that market samples of neoarsphenamine may deteriorate in ampule under ordinary conditions. They may change in color, solubility, toxicity, mobility in ampule, and odor after varying periods.

The change in color in some instances consisted of a slight deepening of the original golden-yellow powder, whereas in other cases a change to a brick red occurred, such as takes place when the powder is exposed to the air for a day or more. Prolonged incubation at 37° C. or heating at 100° C. caused deepening in color.

A relatively large number of samples showed a decrease in solubility; in one lot it occurred within two weeks after manufacture; in other lots from four months to slightly over two years had elasped before the condition was noticed. The decrease in solubility varied as shown by the production of a turbid solution or a solution containing a fine flocculent precipitate with some lots, to that of a coarse suspension with other lots, the coarse suspension in some cases being practically insoluble in water within half an hour. Incubation of the ampules at 37° C., as well as at higher temperatures, hastens the development of a decrease in solubility.

Many lots, although showing a change in color and solubility, did not show an increase in toxicity. Some had become more toxic as well as difficultly soluble. An increase in toxicity was noticed in some lots after about one year, even though no apparent changes in their physical properties had occurred, the increase in some cases being slight enough to permit the preparations to pass the official toxicity tests, whereas other lots which had retained their ready solubility failed to meet the official toxicological requirements.

The change in the mobility of the powder in ampule was usually so pronounced that a solid mass was formed which was frequently adherent to the sides of the ampule. A marked deepening in color usually accompanied the change from a freely mobile powder to the solid form.

The odor of the neoarsphenamines varied greatly. Some lots emitted an odor resembling ether, others emitted a garlicky odor on opening the ampule. Incubation at 37° C. may produce a pronounced ethereal or a garlicky odor. It is interesting to note that ampules from the same lot varied as to the odor emitted on opening the ampule. Ampules which emitted a garlicky odor usually produced incomplete solutions which emitted a similar odor.

Discussion of Results in Tables and Additional Investigations.

The fact that the chemical constitution of neoarsphenamine is still in dispute should be borne in mind in a discussion of these results.

Neoarsphenamine, as previously stated, is usually considered to be sodium 3, 3′-diamino-4, 4′-dihydroxy arsenobenzene-N-methylene sulphinate, and therefore should have an arsenic content of about 30 per cent. In reality, however, the Government regulations require that commercial samples shall have only about 20 per cent arsenic. They must therefore contain either a large amount of by-products or impurities, or diluent, to reduce the arsenic from the theoretical amount to that which exists in commercial samples.

Macallum,[1] states that certain neoarsphenamines consist to a great extent of reaction by-products. Raiziss and Falkow,[2] have recently reported that considerable variation was found upon analysis of a number of samples of neoarsphenamine obtained from three different laboratories. They found that the deviation in the arsenic to nitrogen ratio, which they regard as a comparatively good indicator of the purity of the arsenical component, was greater for the neoarsphenamines than for the arsphenamines which they examined, indicating that neoarsphenamine is not as definite a compound as arsphenamine. In addition, the values which they obtained for the oxygen requirements were in excess of those required merely by the arseno group, the excess being attributed to the presence of uncombined sodium formaldehyde sulphoxylate. They found, furthermore, that the amount of combined sulphoxylate was greater than that necessary for one amino group and less than that calculated for a substitution product with both amino groups closed, from which they concluded that neoarsphenamine may be assumed to be a mixture of both the mono- and di- substituted products.

We must, therefore, regard commercial neoarsphenamine as a mixture rather than as a definite chemical compound. It would seem to be more nearly exact to describe neoarsphenamine as a substance prepared from arsphenamine by means of formaldehyde sulphoxylate, as prescribed by the present Treasury Department regulations.

The causes for the above deterioration, for the most part, can only be conjectured. The occurrence of physical deterioration in some lots within a relatively short time, about two weeks, for example, whereas it required a year or more for other lots to show a similar change, supports the idea of there being essential chemical differences in the

[1] Macallum, A. Douglas, The Examination of Neoarsphenamine: Jour. Amer. Chem. Soc., 1921, vol. 43, p. 643.

[2] Raiziss, George W., and Falkow, M., The Chemistry of Neoarsphenamine and Its Relation to Toxicity: Jour. Biol. Chem., 1921, vol. 16, p. 209.

character of the various lots. But in addition to this cause there are other influences which tend to bring about this condition, chief of which are the influences of age and temperature. Our results indicate that when neoarsphenamine was kept in ampule at ordinary temperature, many of the lots had deteriorated within two years, so that a lot which is over two years old should be looked upon as one requiring careful investigation before being used clinically.

Temperature was found to be a potent factor in hastening the deterioration of neoarsphenamine in ampule. It was found that a lot would remain unchanged longer at a temperature of 18°–20° C. than when kept at a temperature of 37° C. Whereas profound changes may be readily effected in some lots by heating to 100° C. for 20 minutes, other lots may be practically unchanged by such treatment. The gross character of the changes produced by heating in ampule at 100° C. for 20 minutes are the development of a relatively insoluble product emitting an ethereal or garlicky odor, and the production of a relatively immobile powder which may become adherent to the sides of the ampule. These observations suggest that neoarsphenamine should be kept under conditions similar to those required for vaccines and allied biologics, namely, at ice-box temperature rather than at room or higher temperatures, and that some neoarsphenamine is unsuited for use in the Tropics.

The effect of storage (probably heat) is probably further shown by the following observation. During the war our forces in France employed French neoarsphenamine to some extent. Upon return of our forces the stock of French neoarsphenamine, which was forwarded to the medical supply officer at Washington, was referred to the Hygienic Laboratory for examination, and it was found that much of the returned French neoarsphenamine had become insoluble in water and in some cases had deepened in color markedly; also some had become practically immobile in ampule. Inasmuch as a number of lots of this product which had previously been received from other sources were readily soluble in water, it would appear that conditions of storage may have been responsible for the above-mentioned changes. Age may have played a part; but it would seem that it was not the most important factor, as some of the more recent lots were more profoundly affected than the older ones.

A very pertinent question in connection with the foregoing findings is, Does the above deterioration occur only in domestic products? We are led to believe that deterioration may occur in all products, inasmuch as we have found that certain samples of Canadian and French products were likewise insoluble. This together with the observation of Verda,[4] that a sample of German neosalvarsan ob-

[4] Verda, A., Esame fisico-chimico del neosalvarsan: Schweizerische Apoth. Zeit. 1920, Vol. 58, p. 280.

tained from a foreign military store and which was less than two years old had become insoluble, is indeed strong evidence that deterioration is a general phenomenon and may occur in all products.

In order to ascertain the relative occurrence of deterioration in neoarsphenamine, as evidenced by a change to a more difficultly soluble product, all of the lots which complied with the official requirements, received during the period February, 1919, to February, 1920, from manufacturers B and C were tested in July, 1921. Out of the 49 lots received from manufacturer B, 13 were found to be difficultly soluble, whereas out of the 25 lots received from manufacturer C, 8 lots had changed from a readily soluble to a difficultly soluble powder; in other words, about 25 per cent of the lots issued by manufacturer B and about 30 per cent of those issued by manufacturer C had deteriorated in less than three years' time.

The behavior of lot 9, manufacturer B, indicates that incomplete drying of the product before ampuling is a factor in causing the deterioration of this lot. This lot was sent to the Hygienic Laboratory in two shipments. The history of these two shipments as obtained from the manufacturer is as follows:

Shipment 1 was sent to the Hygienic Laboratory on April 30, 1920, after 6 days' drying. On June 3, 1920, analysis showed that the remainder of the batch, which was kept in a vacuum chamber, had lost approximately 2 per cent of volatile matter and was cut accordingly. Shipment 2 was made on June 8, 1920, and differed from shipment 1 only in having lost about 2 per cent in moisture.

On reexamination of these two shipments in June, 1921, the samples from shipment 1 were found to have turned a deep orange red, were difficultly soluble in water, and emitted a strong ethereal odor, whereas samples from shipment 2 had remained practically unchanged in color, solubility, and odor.

Both shipments had been kept under identical conditions while at the Hygienic Laboratory; that is, at storeroom temperature. The occurrence of a strong ethereal odor in shipment 1 and none in shipment 2 suggested that the loss due to drying was probably largely due to volatilized ether. Upon analysis for arsenic, shipment 1 was found to contain 17.96 per cent arsenic; shipment 2, 18.32 per cent.[5]

It is possible that the heat required to seal the ampules may have been sufficient to induce in some lots changes of so slight a nature as to be undiscoverable at first examination, but which progressed during the ageing of the lots. If such is the case, it could be readily obviated by increasing the length of the ampules.

[5]Determined by Mr. C. G. Remsburg, of the Division of Chemistry, Hygienic Laboratory.

The deterioration of neoarsphenamine, as indicated by physical changes, does not necessarily signify that there is a coincident increase in toxicity. This is amply demonstrated by the results in the tables here presented. The relation of toxicity to solubility was formerly considered to be a more intimate one than the present results indicate.[6]

The former conclusion, namely, that a decrease in the solubility of neoarsphenamine was usually accompanied by an increase in its toxicity, was formed from experiments carried out almost wholly using neoarsphenamines from manufacturer D. It is interesting to note, in Table IV (manufacturer D), that out of the 10 lots examined, 3 which had decreased in solubility had also become more toxic. It would appear from the above results that this relationship holds particularly for the products from manufacturer D.

The results of the toxicity tests reported in the tables can not be properly interpreted without taking into consideration the possibility of there being a seasonal variation in the resistance of rats to neoarsphenamine. It has been suspected for some time that rats were more resistant to neoarsphenamine during the summer months than during the winter months. The present results afford some evidence that such is the case. For example, lot 7, manufacturer C, gave a higher mortality rate in March than in June or September of 1921; lots 8 and 10, manufacturer C, and lot 1, manufacturer D, gave higher mortality rates in March than in June of 1921.

Further evidence that a seasonal variation to neoarsphenamine exists in rats is shown by the following data: Compilations made from the records of official tests made during March, 1920, showed that out of 65 lots of neoarsphenamine tested, in which 325 rats were used, 87 rats, or about 27 per cent, died within 7 days; whereas out of 47 lots tested during June, 1920, in which 235 rats were used, 25 rats, or about 11 per cent, died within 7 days. The mortality percentage for March and June, 1921, was, respectively, 45 and 15 per cent. These data would indicate that white rats are considerably more resistant in June than in March.

Considering the above findings on the seasonal variability of the rat, in connection with the results of the toxicity tests given here, it can be concluded that lots 8 and 12, manufacturer A, and lot 10, manufacturer B, had become more toxic, within about a year, without manifesting a coincident change in solubility; whereas lots 2, 8, and 9, manufacturer D, had not only become more toxic but had decreased in solubility as well.

[6] Roth, George B., Some Salient Facts Regarding the Toxicity of Arsphenamine and Neoarsphenamine: Arch. Derm. and Syph., 1920, vol. 2, p. 292.

It is very probable that the changes in toxicity in neoarsphenamine are due to oxidative processes occurring within the ampule. If the changes that occur are of such nature, they would likely be very similar to those described by Hunt[1] recently for aqueous solutions of arsphenamine; that is, that arsphenamine in solution first becomes more toxic upon cleavage of the arseno group and less toxic upon further oxidation of the cleavage products from the trivalent to the pentavalent form. Lots which were found to have decreased in solubility, therefore, may have passed through a more toxic stage during the progress of their deterioration.

Conclusions.

1. Commercial neoarsphenamine is a relatively unstable substance in ampule.

2. Age, heat, and incomplete drying of the substance before ampuling are factors in causing deterioration in commercial neoarsphenamine.

3. The deterioration of neoarsphenamine is shown by changes in color, mobility in ampule, toxicity, solubility, and odor.

4. The results of the experiments suggest (*a*) that inasmuch as neoarsphenamine may deteriorate within a short time after manufacture, and in order to secure further data on its keeping properties, the date of manufacture might be given on the label of all lots issued; (*b*) that neoarsphenamine should be kept under conditions similar to those required for vaccines; that is, at ice-box temperature.

BIOLOGICAL PRODUCTS.

ESTABLISHMENTS LICENSED FOR THE PROPAGATION AND SALE OF VIRUSES, SERUMS, TOXINS, AND ANALOGOUS PRODUCTS.

The following table contains a list of the establishments holding licenses issued by the Treasury Department in accordance with the act of Congress approved July 1, 1902, entitled "An act to regulate the sale of viruses, serums, toxins, and analogous products in the District of Columbia, to regulate interstate traffic in said articles, and for other purposes."

The licenses granted to the following establishments for the products mentioned do not imply an indorsement of the claims made by the manufacturers for their respective preparations. The granting of a license means that inspections of the establishment concerned and laboratory examinations of samples of its products are made regu-

[1] Hunt, Reid, Some Factors Relating to the Toxic Action of Arsphenamine: Jour. Am. Med. Assoc., 1921, vol. 76, p. 854.